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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CAROLINE S. BROWN ART UNIT: 1813

SERIAL NO. : 08/465,747 EXAMINER: Mosher

FILED : June 6, 1995

FOR : HUMAN PARVOVIRUS B19 PROTEINS AND VIRUS-LIKE PARTICLES, THEIR PRODUCTION AND THEIR USE IN DIAGNOSTIC ASSAYS AND VACCINES

INFORMATION DISCLOSURE STATEMENT AND SECOND PRELIMINARY AMENDMENT

Hon Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Submitted herewith is a sworn translation of the priority document to perfect the priority date under 35 USC 119 of September 14, 1989.

Kajigaya et al. and Borisova et al. were cited in Paper No. 13 of the parent application. Borisova et al. was published after the September 14, 1989 priority date and hence is not available as a reference. Kajigaya et al. was published in the October, 1989 issue of PNAs. A copy of Kajigaya et al. is enclosed showing the receipt by the Office of this article in October, 1989. Kajigaya et al. is hence not available as a reference.

WO90/05538 submitted herewith was published May 31, 1990, and therefore is also not available as a reference. However, we call the Examiner's attention to the fact that WO90/05538 claims priority from United States Application Serial No. 270,098, filed November 14, 1988.

The claims now on file overcome all of the rejections

outstanding in Paper No. 13 in the parent application, as discussed below.

I. Sections 101 and 112.

Claims 49-57 are directed to B19 capsid proteins useful as diagnostic agents to detect antibodies to human parvovirus B19 in human sera. We believe that no rejection of the corresponding claims in the parent application was made for lack of utility under 35 USC 101.

Claims 49-57 fully satisfy 35 USC 112. These claims involve no new matter and are fully enabled. We believe that no rejections were made under 35 USC 112 in the parent application to claims corresponding to claims 49-57.

II. VP2 Capsid Protein: Novelty and Utility

As defined by new claims 49-53, the present invention provides isolated and purified VP2 capsid protein of the human parvovirus B19 obtained by recombinant DNA techniques. In particular, the VP2 capsid protein is expressed in and isolated from Spodoptera frugiperda cells by means of a baculovirus expression system. As stated in the Preliminary Amendment filed June 6, 1995, claims 49-53 do not include any new matter. See page 5, lines 6-23, page 17, lines 20-23 and Example 4 of the present application.

Ozawa et al. (T) identified the VP1 and VP2 capsid proteins of the human parvovirus B19 in infected human erythroid bone marrow cells. Ozawa et al. did not disclose isolated and purified VP2 capsid protein as in claims 49 and 50. They did not

disclose recombinant VP2 capsid protein as in claims 51-53. Hence claims 49-53 are novel under 35 USC 102.

Claims 49-53 fully satisfy 35 USC 101 and 35 USC 112, first paragraph, since the VP2 capsid protein is disclosed as useful in the detection of B19-specific antibodies. See page 7, line 22 to page 8, line 7 and Example 4.

III. VP1 Capsid Protein: Novelty and Utility

As defined by new claims 54-57, the present invention also provides isolated and purified VP1 capsid protein. The VP1 capsid protein is obtained by recombinant DNA techniques and may be coexpressed with VP2 capsid protein in Spodoptera frugiperda cells using a baculovirus expression vector system.

Claims 54-57 do not include new matter. See Example 3, page 5, lines 6-23 and page 18, lines 1-2 of the present application.

Claims 54-57 are not anticipated by Ozawa et al. (T), because Ozawa et al. did not disclose isolated and purified VP1 capsid protein obtained by recombinant DNA techniques. Page 7, line 22 to page 8, line 7 and Example 2 describe the use of the VP1 capsid proteins of the present invention in the detection of B-19 specific antibodies.

IV. THE PRIOR ART, TAKEN AS A WHOLE, DOES NOT DISCLOSE OR SUGGEST ANY UTILITY FOR THE UNFUSED VP1 AND VP2 CAPSID PROTEIN AND HENCE PROVIDES NO MOTIVATION FOR THE EXPRESSION, ISOLATION AND PURIFICATION OF THE B19 CAPSID PROTEINS OF THE PRESENT INVENTION

A. SUMMARY OF ARGUMENT

The primary references, Ozawa et al. (T), Sisk et al. and Cotmore et al. (1986), and the secondary references, Wood et al.

and Mazzara et al., are discussed in detail below. The other references relied on in Paper No. 13 are either irrelevant or redundant. As stated above, Kajigaya et al. and Borisova et al. are removed as references.

The prior art teaches only the fusion expression of B19 capsid proteins, or segments thereof, as a fusion protein and teaches away from unfused VP1 and VP2 capsid protein. The prior art relating to canine parvovirus is irrelevant, because of the absence in the record of evidence showing close homology between canine and human parvovirus. In fact, there is no such homology.

Claims 49-53 define VP2 capsid protein of human parvovirus B19, which forms particles having great similarity as to diameter and morphology to the human parvovirus B19 capsid. See Example 4 and particularly page 17, lines 19-22 of the present application. Nothing in the prior art previously of record or the newly submitted prior art would have led the skilled worker to expect that the VP2 capsid protein of the present invention forms particles that could or would have similar morphology and diameter as the native form of the parvovirus capsid, and hence would have conformational epitopes of the paravovirus capsid.

The VP1 and VP2 capsid proteins of the present invention unexpectedly give no false positives and no false negatives when used to detect B19 antibodies in human sera. See Tables 1 and 2 of the present application. Even if the prior art gives rise to a prima facie case of obviousness for claims 49-57 - which it does not - the unexpected results of the present invention overcome any such inference of obviousness.

B. THE PRESENT INVENTION IS NOT OBVIOUS FROM OZAWA ET AL., COTMORE ET AL. AND SISK ET AL., ALL OF RECORD

Ozawa et al. (T) of record cannot be viewed in isolation. Rather, the prior art as a whole must be compared to the present invention in order to determine the patentability of claims 49-57. In particular, Ozawa et al. (T) must be considered with Cotmore et al. (1986) and Sisk et al., of record, and with Collett et al., Reviews In Medical Virology, 4: 91-103 (1994) newly cited by applicant, copy enclosed.

It is beyond dispute that Ozawa et al. neither isolated nor purified VP2 (or VP1) capsid protein of the human parvovirus B19 nor disclosed or suggested any use for such VP2 (or VP1) capsid protein. Cotmore et al. and Sisk et al. do not provide the bridge between Ozawa et al. and the present invention.

As described in Cotmore et al. (1986), page 555, left column, and as explained in paragraph 4 of the enclosed Declaration of Dr. Spaan, Cotmore et al. expressed in E. Coli. a "tripartite fusion protein" and a "bipartite fusion protein" each containing a B19 capsid protein fragment consisting of 284 amino acids of the VP1 capsid protein (namely amino acids 151-435 of VP1, as shown in the diagram attached to Dr. Spaan's Declaration). In the "tripartite fusion protein", the B19 fragment was fused between lambda repressor protein and beta-galactosidase whereas in the bipartite fusion protein, the beta-galactosidase was deleted. In both fusion proteins, however, the B19 fragment was just that, a mere fragment of a B19 capsid protein. Cotmore et al. thus failed to express any fused,

complete VP1 or VP2 capsid protein. Cotmore et al. certainly failed to produce any unfused VP1 or VP2 capsid protein.

Moreover, Cotmore et al. (1986) did not use their fusion proteins to detect B19 antibodies in any sample, much less in human sera. Nor did Cotmore et al. even suggest,much less disclose, that VP1 or VP2 capsid proteins could be used to detect B19 antibodies.

Cotmore et al. (1986) is thus irrelevant to the present invention. This is confirmed by Sisk et al., at page 1079, right column, second paragraph under "DISCUSSION", who addresses a major shortcoming of Cotmore et al.:

> The recent study by Cotmore et al. described the construction of two expression plasmids containing B19 viral DNA sequences encoding the non-structural and capsid proteins of the viral genome. These constructs expressed viral specific B-galactosidase hybrid proteins in E. coli. Rabbit antisera directed against the hybrid protein from the clone expressing nt 2897-3479 recognize both the 83 kD and 58 kD B19 capsid polypeptides in the plasma of acutely infected patients. . . . . However, it was not demonstrated that the expressed recombinant polypeptides produced from these clones could be used as a source of antigen to detect antibodies to parvovirus in the sera of infected patients or from the sera of asymptomatic individuals. (Emphasis added.)

It is submitted that those skilled in the art would have discarded the fusion protein of Cotmore et al. (1986) as a possible diagnostic agent for detecting B19 antibodies.

Sisk et al. also expressed a fusion protein in E. coli. Sisk et al.'s fusion protein (196 KD) consisted of beta-galactosidase and a polypeptide encoded by the structural gene

encoding capsid proteins VP1 and VP2. This fusion protein was stated to be recognized by B19 antibodies present in B19 positive sera, but yet the fusion protein detected antibodies in only 22 out of the 50 sera (44%) obtained from normal healthy adults. Sisk et al. surmise, page 1079, right column, first sentence under "DISCUSSION", that their beta-galactosidase - B19 fusion protein was "a reliable source of viral antigen for detection of B19 related antibodies in human serum". Those skilled in the art would not readily agree. See ¶ 5 of the Spaan Declaration.

It is significant that neither Sisk et al. nor Cotmore et al. (1986) state that their fusion proteins had the native conformation of the B19 capsid. The skilled worker would know that the fusion partner would most likely prevent the fusion protein from folding to the precise structure of the B19 capsid protein. See ¶ 6 of Dr. Spaan's Declaration. The skilled worker would thus have cause to doubt whether B19 capsid proteins produced as fusion proteins would have all of the native conformational epitopes of the B19 capsid. In fact, the enclosed Collett et al., explains that recombinant fusion proteins expressed in E. coli lack the native conformation of the B19 capsid. See page 98, left column. Mentioned among these fusion proteins are those of Cotmore et al. and Sisk et al. The skilled worker would thus have cause to doubt whether such fusion proteins would reliably detect B19 antibodies in human sera. See ¶7 of Dr. Spaan's Declaration.

In fact, the Sisk et al. fusion protein would have been recognized by those skilled in the art as giving rise to numerous

false negatives. Thus, as explained in ¶ 8 of Dr. Spaan 's Declaration, about 70-80% of human sera contain B19 antibodies. See also page 15, lines 32-36 of the present application where it is reported that 76% of randomly selected donors were positive. Since the Sisk et al. fusion protein gave only 44% positives, those skilled in the art would have known that these fusion proteins failed to detect a large percentage of positives. As to Cotmore et al. (1986), since they provided no data in detection of B19 antibodies in human sera, their work does not survive more than minimal scrutiny. See ¶9 of the Spaan Declaration.

In addition, Sisk et al. do not disclose or suggest the use of recombinant unfused VP1 and/or VP2 capsid proteins to detect antibodies to human B19 virus in human serum. To the contrary, at page 107, right column, first paragraph under "DISCUSSION", Sisk et al. actually teach away from the use of recombinant unfused VP1 and/or VP2 capsid proteins by discussing the advantages of recombinant fusion proteins and the disadvantages of recombinant unfused proteins:

> The B-galactosidase fusion protein produced by pWPS17.1 in E. coli was shown to be a reliable source of viral antigen for detection of B19 related antibodies in human serum. There have been numerous reports of using B-galactosidase fusion proteins as a source of antigen from a variety of different viruses. For Western blot analysis the resolution of high molecular weight fusion proteins in a region of the polyacrylamide gel devoid of other E. coli proteins is a distinct advantage. In addition, protein segments produced as B-galactosidase fusions are generally stable while the same segments unfused to B-galactosidase are often degraded in E. coli. The ability to construct

> prokaryotic fusion proteins is advantageous when the sole source of a viral antigen is the live virus. (Emphasis added).

One skilled in the art on September 14, 1989, the effective date of the present application, would not start from Ozawa et al. alone, but rather would consider the totality of the prior art at that time relating to human parvovirus B19, namely Ozawa et al., Sisk et al. and Cotmore et al. (1986). These references as a whole teach that recombinant fusion proteins of beta-galactosidase and B19 capsid protein may be capable of detecting antibodies to human B19 virus in human serum to some extent, but that recombinant unfused B19 capsid proteins would be unlikely to be useful at all. Further, the skilled worker would not be convinced that the sensitivity of a B19 capsid protein in the form of a fusion protein would be sufficiently reliable. Certainly, none of the Ozawa, Cotmore et al. (1986) and Sisk et al. references, of record, discloses or suggests the surprising effect that B19 VP2 capsid protein expressed in baculovirus/insect cell culture is able to form virus-like particles having the diameter and morphology of native B19 virus. See page 17, lines 20 to 22 of the present application.

Nor do these references disclose or suggest that the VP1 and VP2 capsid proteins of the invention provide a diagnostic agent for detecting B19 antibodies in human sera that gives no false positives and no false negatives. The data in Tables 1 and 2 of the present application show that the VP1 and VP2 capsid proteins of the present invention gave no false positives and no false negatives when used to detect B19 antibodies in human sera. See

also, the immunofluorescence studies reported in Brown et al., Virus Res., 15: 197-212 (1990), copy enclosed, which likewise show no false negatives or false positives. It is surely a principle of diagnostic assays that the ideal assay gives no false positives and no false negatives. Given the state of the prior art, it was surprising and unexpected that the VP1 and VP2 capsid proteins of the present invention could provide this highly advantageous result. See ¶10 of the Spaan Declaration.

C. The Prior Art Relating To Canine Parvovirus Is Irrelevant

Wood et al. disclose the expression of the canine parvovirus VP2 capsid protein and its use as a vaccine against canine parvovirus. Mazzarra et al. disclose the coexpression of the canine parvovirus VP1 and VP2 capsid proteins and its use as a vaccine against canine parvovirus. Wood et al. is utterly silent as regards human B19 capsid proteins, while Mazzarra et al. only contains the speculative statement that their method of expression could be used to produce empty capsid proteins "of other types of parvovirus including feline, mink, porcine, bovine and human parvoviruses". These references, alone or together with the other references discussed above, do not disclose or suggest the present invention.

There is no evidence of record that canine parvovirus and human parvovirus are sufficiently homologous to lead the skilled worker to expect either a) that one could obtain empty viral capsids of B19 parvovirus using the methods of Wood et al. or Mazzarra et al. or b) that such B19 capsids would be useful for

any purpose. In fact, canine parvovirus and human B19 parvovirus are structurally quite dissimilar.

Enclosed is a copy of Chapman et al., Virology, 194, 491-508 (1993), which discusses an alignment of parvovirus capsid sequences against the sequence and three-dimensional structure of CPV. As stated in ¶ 11 of Dr. Spaan's Declaration, the sequence similarity between CPV and B19 was highest for the conserved structural motif which forms the inner core of the particle. The overall identity was on the icosahedral three fold axes, which contain residues involved in host range and antigenicity. Regarding the surface features, Dr. Spaan notes at ¶11 of his/her Declaration that there was a close but inexact correspondence of B19 antigenicity and CPV surface accessibility suggesting that the general region is antigenic but that the structures are likely to differ in detail.

Also enclosed is a copy of Agbande et al., Virology, 203, 106-115 (1994), which describes the structure of baculovirus-produced VP2 capsids at a fairly low resolution, 8 Angstroms. As stated in ¶12 of Dr. Spaan's Declaration, this means that only very large structural features can be distinguished, such as the inner core of the particle. To obtain more information, the B19 structure map was compared to that of the high resolution map of feline parvovirus (FPV) to which CPV is almost identical, which demonstrated the similarity between these inner core structures. However, this similarity is unimportant because the inner core structure is conserved in most icosahedral viruses infecting eukaryotes, and it is the surface features that are important in

a diagnostic assay, since the epitopes are on the surface. Dr. Spaan notes (¶12) that the comparison between B19 and FPV showed striking differences in the surface topology as, in the B19 capsids, the prominent three fold spikes were absent (see the gap in the sequence alignment of Fig. 6 between residues 415-435; this figure was adapted from the alignment of the enclosed Chapman et al., Fig. 3; see also Fig. 8 in the enclosed Agbandje paper). Other surface differences which were difficult to interpret have also been predicted by Chapman et al. (insertions and deletions in the sequence alignment).

Cotmore et al. (1986), of record, also notes at page 548, right column, that

> B19 is a parvovirus, but DNA hybridization studies have showed that it is only very distantly related to other serotypes in this family.

citing Cotmore et al., Science, 226: 1161-1165 (1984), copy enclosed.

Further, Cossart et al., The Lancet, January 11, 1995, 72-73, copy enclosed, show that canine parvovirus and human B19 parvovirus do not cross-react antigenically. (¶13 of the Spaan Declaration.)

Given that there is no cross-reaction antigenically between canine parvovirus and human B19 parvovirus and that there is low sequence homology and low DNA homology between canine parvovirus and human B19 parvovirus, the skilled worker would have no basis to extrapolate the work done by Wood et al. and Mazzarra et al. on the use of CPV as a vaccine to the expectation that B19 capsid

proteins would be useful as vaccines, much less that they would be useful as diagnostic agents for reliably detecting b19 antibodies in human sera. See ¶14 of Dr. Spaan's Declaration.

In summary, Cotmore et al. (1986) of record and Sisk et al. disclose fusion proteins containing amino acid sequences of B19 capsid proteins. While these could possibly be of limited value in a Western blot analysis, which denatures the protein and exposes epitopes, they would not be of value in a more sensitive type of assay, such as ELISA, where the three-dimensional structure of the protein must be preserved. See ¶6 of the Spaan Declaration. See ¶6 of the Spaan Declaration. Certainly, Cotmore et al. (1986) and Sisk et al. do not disclose or suggest the unfused capsid proteins of the present invention. Nor do they provide any motivation to the skilled worker to make the unfused proteins.

Wood et al. and Mazzarra et al. do disclose unfused canine parvovirus capsid proteins, but due to the lack of DNA and structural similarity between canine parvovirus and human B19 parvovirus, the skilled worker would not have had any basis for predicting whether unfused human B19 capsid proteins could be formed, and, if formed, whether they would have any utility whatsoever. Certainly, Wood et al. and Mazzarra et al. fail to

disclose or suggest the B19 capsid proteins of the present invention, which have the native conformation of the B19 capsid.

To complete the record of the above application, we enclose a copy of Form PTO-1449 listing the references submitted herewith. Also enclosed are the five Forms PTO-892 from the parent application Serial No. 07/838,715, from which the present application claims priority under 35 USC 120. Please make all of the references listed in Forms PTO-1449 and PTO-892 of record.

Allowance is requested.

Respectfully submitted,

CAROLINE S. BROWN

By: ROSENMAN & COLIN LLP

Per: _____

Jesse D. Reingold
Reg. No. 20,461
Attorney for Applicants

575 Madison Avenue
New York, New York 10022
(212) 940-8800